

Message

From: Conley, Justin [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=8E08A48A85D141DBA00D8377D89E9A3E-CONLEY, JUS]
Sent: 2/27/2019 1:22:43 PM
To: McCord, James [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=McCord, James]
Subject: RE: liver samples

Just wanted to be sure about the meeting this morning – we're just getting together to talk about the sample prep and instrument loading procedures and when that can happen, right? Like, we don't need to bring the whole set of samples with us, do we?

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Postdoctoral Toxicologist
U.S. Environmental Protection Agency
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(919) 541-3326

From: McCord, James
Sent: Wednesday, February 20, 2019 11:00 AM
To: Conley, Justin <Conley.Justin@epa.gov>; Strynar, Mark <Strynar.Mark@epa.gov>
Subject: RE: liver samples

I realize I said we should get together this week, but I forgot that we have instrument training T/W/Th and the week was shortened. Can we push to sometime next week?

--

James McCord

From: Conley, Justin
Sent: Wednesday, February 13, 2019 9:49 AM
To: McCord, James <mccord.james@epa.gov>; Strynar, Mark <strynar.mark@epa.gov>
Subject: RE: liver samples

Sounds good. A sample list is attached with serum and liver samples separated. We estimated the concentrations based on the previous analyses you guys have done. Let me know how if you have any questions about the list and how you want to proceed.

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(919) 541-3326

From: McCord, James
Sent: Tuesday, February 12, 2019 4:17 PM
To: Strynar, Mark <strynar.mark@epa.gov>; Conley, Justin <Conley.Justin@epa.gov>
Subject: RE: liver samples

If you send me the sample list I can plot out the runs and show you how to prep the samples sometime early next week, it is actually fairly straightforward.

--

James McCord

From: Strynar, Mark

Sent: Tuesday, February 12, 2019 4:16 PM

To: Conley, Justin <Conley.Justin@epa.gov>

Cc: McCord, James <mccord.james@epa.gov>

Subject: RE: liver samples

Justin,

So James and I are not the bottle neck, we can discuss you helping out. We don't do anything magical and it would take a rock off of our pile. As you have an analytical chem background that is even better.

Mark

From: Conley, Justin

Sent: Tuesday, February 12, 2019 3:43 PM

To: Strynar, Mark <strynar.mark@epa.gov>

Cc: McCord, James <mccord.james@epa.gov>

Subject: RE: liver samples

I figured you were slammed. Our timeline is just the typical "sooner is better". I have talks coming up that I would include the data if we had it, and we plan to write up the study by this fall so we'd include this data if we have it as well. It's just really useful information for those in the risk assessment community who are interpreting our toxicity data. I'm happy to learn how to prep the samples and get them ready for analysis if you think that would actually help. For what it's worth I do have a background in analytical chem but haven't been in that wheelhouse in quite a while.

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From: Strynar, Mark

Sent: Tuesday, February 12, 2019 7:32 AM

To: Conley, Justin <Conley.Justin@epa.gov>

Cc: McCord, James <mccord.james@epa.gov>

Subject: RE: liver samples

Justin,

We are overwhelmed with catching up with state and Regional support. When you bring the samples over they will simply sit in the freezer until we get to them. The work involved to run 103 samples or a smaller set is not that different. Thus I think it best to run all samples rather than a select set usually. Let us know what your timeline is and

when we need to get these done by and we can figure it out. It may even involve getting you to learn how to run the method and get samples onto the mass spectrometer.

Mark

From: Conley, Justin
Sent: Thursday, February 07, 2019 3:32 PM
To: Strynar, Mark <strynar.mark@epa.gov>
Subject: RE: liver samples

Mark,

You had mentioned that we should get our samples to you around the end of Feb and I'm just trying to get stuff organized and prioritized. We have 103 liver samples and 103 serum samples. That's a lot. If you can do them all, great. If that's way too many and we need to trim it down to the most important ones, that's not a problem. Just wanted to get your thoughts.

Cheers,

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From: Strynar, Mark
Sent: Wednesday, February 06, 2019 8:22 AM
To: Conley, Justin <Conley.Justin@epa.gov>
Subject: RE: liver samples

It is the right one and we received one. I will send the packing slip asap.

Mark

From: Conley, Justin
Sent: Wednesday, February 06, 2019 8:19 AM
To: Strynar, Mark <strynar.mark@epa.gov>
Subject: RE: liver samples

Weird, Christy never received the invoice or shipping notice, but glad you got it. It's the correct IS, right? I think she only got approval for one unit (not even sure how much you got), but if you need more I'm sure we can order another.

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From: Strynar, Mark
Sent: Wednesday, February 06, 2019 7:14 AM
To: Conley, Justin <Conley.Justin@epa.gov>
Subject: RE: liver samples

Good to know because a package of GenX IS showed up yesterday for me. I was not sure why.

Mark

From: Conley, Justin
Sent: Tuesday, February 05, 2019 1:54 PM
To: Strynar, Mark <strynar.mark@epa.gov>
Subject: RE: liver samples

Thanks Mark. I think we're going to look into getting some from Synquest and maybe combine the order with Johnsie.

Christy put in an order for the GenX IS and she put the delivery information to have it sent directly to you. I think with orders being all screwed up from the shutdown that she hasn't gotten confirmation, but when she does I'll let you know.

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From: Strynar, Mark
Sent: Monday, February 04, 2019 4:13 PM
To: Conley, Justin <Conley.Justin@epa.gov>
Subject: RE: liver samples

For Nafion BP2 we may have a source from a synthesis for UNC. How much would you need? In addition I am told the Synquest is synthesizing Nafion BP2 for Rebecca Fry at UNC for her studies (5 g I think). They are a commercial source but I am not sure if we can get some.

For the Nafion BP1 I don't know of a source.

From: Conley, Justin
Sent: Monday, February 04, 2019 1:29 PM
To: Strynar, Mark <strynar.mark@epa.gov>
Subject: RE: liver samples

Mark,

Another quick question – we're interested in doing some rat studies with Nafion Byproduct 2 (or Byproduct 1), are there any chemical vendors supplying this compound yet that you know of?

Justin Conley
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From: Strynar, Mark
Sent: Thursday, January 31, 2019 8:16 AM
To: Conley, Justin <Conley.Justin@epa.gov>
Subject: RE: liver samples

Justin,

Digging out of the hole still the furlough left. We can fit in some liver and serum for GenX but I am guessing not until the end of the month. Also we could begin to think about the PFMOAA in serum effort at the same time.

One thing I am beginning to really be squeezed on is proper QA documentation for the projects I am working on with all things involved with PFAS. We may need to sit down with my QA people and yours to head this off at the pass if they thing we are not currently adequately covered.

Mark

From: Conley, Justin
Sent: Tuesday, January 29, 2019 2:31 PM
To: Strynar, Mark <strynar.mark@epa.gov>
Subject: RE: liver samples

Hey Mark,

Hope you made the best of the shutdown. I just wanted to touch base and see how backlogged you are and what your schedule looks like moving forward. We have quite a few liver and serum samples that could be analyzed for GenX, as we also now have some rat serum that could be analyzed for PFMOAA, if possible.

I'm sure you're crazy busy and have a full schedule but wanted to get a ballpark idea of when we might be able to squeeze some samples in to you, or even if you're still interested. We've been holding off on buying the GenX IS but will still proceed with that if you think you'll be able to work with us.

Cheers,

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From: Strynar, Mark
Sent: Thursday, December 13, 2018 8:47 AM
To: Conley, Justin <Conley.Justin@epa.gov>

Cc: Lambright, Christy <Lambright.Christy@epa.gov>; Gray, Earl <Gray.Earl@epa.gov>

Subject: RE: liver samples

Justin,

Any samples will have to wait until after the new year. I am still catching up with other water commitments. No rush to get them over here.

Mark

From: Conley, Justin

Sent: Tuesday, December 11, 2018 11:51 AM

To: Strynar, Mark <strynar.mark@epa.gov>

Cc: Lambright, Christy <Lambright.Christy@epa.gov>; Gray, Earl <Gray.Earl@epa.gov>

Subject: RE: liver samples

Hi Mark,

Hope you enjoyed the winter weather.

Christy got approval to order a vial of IS and she is planning on just having it shipped here to you, unless you say otherwise and would like us to receive it and then bring it to you. We might be able to order a second vial if needed in the future.

Last week we ran another complete block of short term (5d) dosing with GenX. It was the same protocol and dose range as the previous set of samples you just ran for us. This time we collected a piece of maternal liver and a single piece of fetal liver (as opposed to two male and two female pieces we collected last time). We were also able to collect fetal serum.

We'd like to bring you the maternal and fetal liver homogenates (n=30) and maternal and fetal serum samples (n=30) to analyze if you are able. The serum from this experiment could hopefully be run along with the maternal serum from the previous set of samples we brought you that you weren't able to run. Then we would have a pretty robust set of matched liver and serum concentrations for the mom and the fetus across a pretty sizable dose range.

Let me know what works for you. I can bring them over to you anytime.

Cheers,

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From: Strynar, Mark

Sent: Wednesday, November 28, 2018 7:41 AM

To: Conley, Justin <Conley.Justin@epa.gov>

Subject: RE: liver samples

Justin,

That is the only IS available so yes that is the one. I think one should be fine, however if you are inclined to get two just incase we run low or out I don't think that is a bad idea.

Mark

From: Conley, Justin
Sent: Wednesday, November 28, 2018 7:23 AM
To: Strynar, Mark <strynar.mark@epa.gov>
Subject: Re: liver samples

Mark,

We are making plans to order a vial of GenX IS from Wellington in the next few weeks. Just wanted to double check with you to make sure that was still the IS you use and that 1 vial will be sufficient.

Hope you had a nice Thanksgiving.

Cheers,

Justin

From: Strynar, Mark
Sent: Thursday, November 1, 2018 3:50 PM
To: Conley, Justin
Subject: RE: liver samples

Justin,

The only vendor for GenX IS is Wellington Labs (see page 155). I think one vial would do the job.

The item is called M3HFPO-DA

<https://www.well-labs.com/wellingtoncatalogue1618.html>

Mark

From: Conley, Justin
Sent: Thursday, November 01, 2018 2:55 PM

To: Strynar, Mark <Strynar.Mark@epa.gov>; McCord, James <mccord.james@epa.gov>

Cc: Gray, Earl <Gray.Earl@epa.gov>

Subject: RE: liver samples

Great!

We most likely would be able to buy some IS if you guys need us to. Would just need info on vendor, volume, cost, time, etc.

We'll go ahead and collect the tissues and serum along with our other endpoints in the next experiment and be in touch.

Cheers,

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From: Strynar, Mark

Sent: Thursday, November 01, 2018 12:09 PM

To: Conley, Justin <Conley.Justin@epa.gov>; McCord, James <mccord.james@epa.gov>

Cc: Gray, Earl <Gray.Earl@epa.gov>

Subject: RE: liver samples

Justin and Earl,

Interested for sure. Time is the biggest deciding factor. We will need to fit this into our work queue. Our biggest dilemma is having to do multiple assays for serum and liver as you have very large dose ranges. The Liver is really only control or dosed. For the serum we have 3 assays to cover your dose ranges. If you can give us some time to get to them we can do the assays.

We also may need to ask you to purchase some 13C labeled GenX. We have a limited supply and use quite a bit for these assays.

Mark

From: Conley, Justin

Sent: Thursday, November 01, 2018 11:08 AM

To: Strynar, Mark <Strynar.Mark@epa.gov>; McCord, James <mccord.james@epa.gov>

Cc: Gray, Earl <Gray.Earl@epa.gov>

Subject: RE: liver samples

We have analyzed the data many different ways using both untransformed and log transformed values, looking at male versus female fetuses both ignoring and accounting for litter effects, as well as comparing male versus female concentration within each litter, and then the relationships between fetus and mother.

Ex. 5 Deliberative Process (DP)

Attached is a copy of a SAS analysis if you're interested in looking at it.

We have another experiment the first week of December where we plan to use the same dosing design (basically exact replica of prior experiment). Given that it took a great deal of time during the last necropsy to collect the livers from two male and two female fetuses for each litter, and there appears to be no major difference between male/female concentrations, but there is a maternal versus fetal difference - we're thinking of collecting another maternal liver sample and then just a single fetal liver sample from each litter. The other important piece of the puzzle would be having the serum concentrations for both the moms and fetuses to relate to the liver concentrations.

All together, this would be 30 more liver samples (1 mom and 1 fetus per litter), and a total of 45 serum samples (1 mom and 1 fetus per litter, plus the 15 maternal serum samples we gave you from the last experiment).

Are you guys interested in this? Have the time?

We believe it's pretty interesting since our hypothesis is the liver is the primary target organ for toxicity. It would make for a good story if the serum is lower in the fetus but for some reason the liver is higher than the moms. It would also help explain the gene expression profiles we have observed.

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From: Strynar, Mark
Sent: Monday, October 29, 2018 7:46 AM
To: Conley, Justin <Conley.Justin@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Ex. 5 Deliberative Process (DP)

Mark

From: Conley, Justin

Sent: Friday, October 26, 2018 3:47 PM

To: Strynar, Mark <Strynar.Mark@epa.gov>; McCord, James <mccord.james@epa.gov>

Cc: Gray, Earl <Gray.Earl@epa.gov>

Subject: RE: liver samples

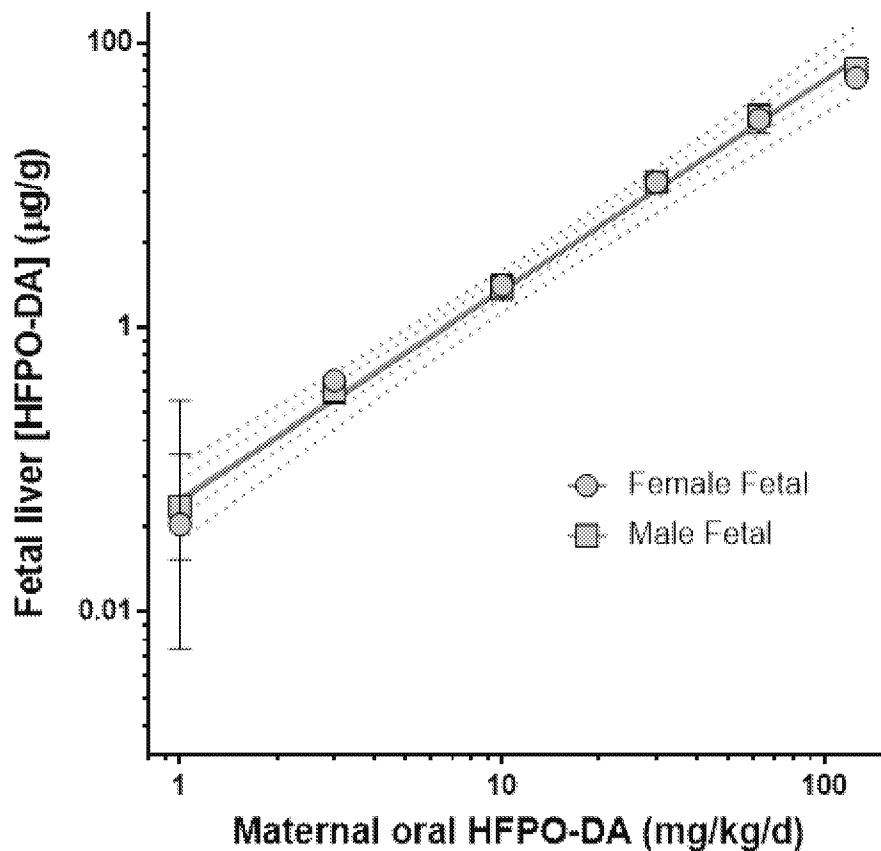
Ex. 5 Deliberative Process (DP)

Ex. 5 Deliberative Process (DP)

We'll touch base

next week after some more number crunching. Thanks again and have a great weekend.

Transform of Fetal Liver GenX MvF - Litter means



Are the slopes equal?

$F = 0.002936$, $DFn = 1$, $DFd = 8$

$P = 0.9581$

Are the elevations or intercepts equal?

$F = 0.1417$, $DFn = 1$, $DFd = 9$

$P = 0.7153$

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From: Strynar, Mark
Sent: Friday, October 26, 2018 11:02 AM
To: Conley, Justin <Conley.Justin@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Here is the data and a brief writeup of what we did. Let me know if you find anything that seems off. James has also been looking at the data on our end with some stats packages. Let us know what you see. For the first 3 doses (1, 3, 10 mg/kg) the maternal livers do not seem to differ. Then you have a dose response curve.

Mark

From: Conley, Justin
Sent: Friday, October 26, 2018 9:40 AM
To: Strynar, Mark <Strynar.Mark@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Hi Mark,

Just checking back in. We have another short term in utero study scheduled for dosing in early Dec that is intended to be a repeat block of the prior study which is where the liver samples came from (same age maternal rats and same dosing regime to increase sample size). We are working out exactly what endpoints to collect and based on the results of the liver samples we could either collect more (if you have interest/time to analyze them) or decide what we have is good enough and do something else. Once we see what you guys found we can make some decisions.

Cheers,

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From: Strynar, Mark
Sent: Tuesday, October 16, 2018 10:03 AM
To: Conley, Justin <Conley.Justin@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

We ran the controls and are about to embark on the dosed maternal/fetal livers today.

Mark

From: Conley, Justin
Sent: Tuesday, October 16, 2018 9:35 AM
To: Strynar, Mark <Strynar.Mark@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Hi Mark,

Just wanted to touch base and see if you had a chance to run those liver samples.

Cheers,

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From: Strynar, Mark
Sent: Wednesday, September 26, 2018 3:41 PM
To: Conley, Justin <Conley.Justin@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Works fine for me. My office is D 285.

Mark

From: Conley, Justin
Sent: Wednesday, September 26, 2018 3:17 PM
To: Strynar, Mark <Strynar.Mark@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

No worries, sounds good. Does 8am work?

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From: Strynar, Mark
Sent: Wednesday, September 26, 2018 3:14 PM
To: Conley, Justin <Conley.Justin@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Justin,

I have been tied up all day today. How about tomorrow AM? I get in at 7 AM.
Mark

From: Conley, Justin
Sent: Tuesday, September 25, 2018 2:02 PM
To: Strynar, Mark <Strynar.Mark@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Sounds good, just let me know when works for you, I can bring them by about any time

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From: Strynar, Mark
Sent: Tuesday, September 25, 2018 1:36 PM
To: Conley, Justin <Conley.Justin@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

I think tomorrow would be better. I am pretty tied up today.

Mark

From: Conley, Justin
Sent: Tuesday, September 25, 2018 1:06 PM
To: Strynar, Mark <Strynar.Mark@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Great, got them all ready. Should I bring them over this afternoon sometime?

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From: Strynar, Mark
Sent: Tuesday, September 25, 2018 8:35 AM
To: Conley, Justin <Conley.Justin@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

The method is similar but I will do serum and liver separate. The calibration curve will be run with blank serum or blank liver individually.

Mark

From: Conley, Justin
Sent: Monday, September 24, 2018 3:18 PM
To: Strynar, Mark <Strynar.Mark@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Great. We have the livers homogenized now. Is your method for prepping and analyzing liver samples similar to serum? I'm asking because if it's convenient for you I can also thaw out the serum samples we collected from the moms and take an aliquot from each to add to the liver samples (this would be 15 more samples for a total of 90).

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From: Strynar, Mark
Sent: Monday, September 24, 2018 11:56 AM
To: Conley, Justin <Conley.Justin@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Justin,

Just give me the fresh homogenates. I will do the rest.

Mark

From: Conley, Justin
Sent: Monday, September 24, 2018 10:24 AM
To: Strynar, Mark <Strynar.Mark@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Sounds good. We can homogenize, then do you just want me to pull out 25uL aliquots from each and bring those to you?

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From: Strynar, Mark
Sent: Monday, September 24, 2018 9:40 AM
To: Conley, Justin <Conley.Justin@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Justin,

As long as you know the mass of liver and volume of water added for each don't adjust anything. We can deal with that analytically. Just homogenize them best you can. We then need to subsample 25 uL before freezing for analysis.

Mark

From: Conley, Justin
Sent: Monday, September 24, 2018 8:27 AM
To: Strynar, Mark <Strynar.Mark@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Hi Mark,

Thanks for getting back. That's exactly what I was wondering, but I didn't know what to do at the time last Friday after we completed the necropsy and had the samples collected. I basically sorted the samples into bins based on the sample weight and added a fixed volume of HPLC grade water (e.g., samples that were ~35mg got 100uL water, samples ~40mg got 120uL etc). They are in the fridge in our lab at the moment. What

do you suggest we do? Try to adjust the water volume in each of them to be exactly 3:1? They have not been homogenized yet.

Cheers,

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From: Strynar, Mark
Sent: Monday, September 24, 2018 7:48 AM
To: Conley, Justin <Conley.Justin@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: RE: liver samples

Justin,

I was off on Friday. I can give you our lab DI water we use and you can add and homogenize 3:1 water to liver with a physical homogenizer (polytron. We could then subsample 25 uL of the homogenate for analysis like we do with serum. We like to add the water exactly 3:1 so if for example you had 53.2 mg of liver we would add $53.2 * 3 = 159.6$ mg of water as 159.6 uL of the DI.

Mark

From: Conley, Justin
Sent: Friday, September 21, 2018 11:43 AM
To: Strynar, Mark <Strynar.Mark@epa.gov>; McCord, James <mccord.james@epa.gov>
Cc: Gray, Earl <Gray.Earl@epa.gov>
Subject: liver samples

Hi Guys,

We just wrapped up the necropsy. We collected ~30-60mg samples of liver from moms and two male and two female fetuses per mom for a total of 75 samples. We have exact masses on each liver sample and they are just in 1.5mL tubes on the benchtop now. What should I do with them? You said to add water at 3:1 ratio – should that be ultrapure/HPLC grade water or just distilled/deionized? How exact does it need to be?

You can just call me too if that's the easiest (number below).

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